

Toward using $\delta^{13}\text{C}$ of ecosystem respiration to monitor canopy physiology in complex terrain

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Abstract In 2005 and 2006, air samples were collected at the base of a Douglas-fir watershed to monitor seasonal changes in the $\delta^{13}\text{CO}_2$ of ecosystem respiration ($\delta^{13}\text{C}_{\text{ER}}$). The goals of this study were to determine whether variations in $\delta^{13}\text{C}_{\text{ER}}$ correlated with environmental variables and could be used to predict expected variations in canopy-average stomatal conductance (G_s). Changes in $\delta^{13}\text{C}_{\text{ER}}$ correlated weakly with changes in vapor pressure deficit (VPD) measured 0 and 3–7 days earlier and significantly with soil matric potential (ψ_m) (P value <0.02) measured on the same day. Midday G_s was estimated using sapflow measurements (heat-dissipation method) at four plots located at different elevations within the watershed. Values of midday G_s from 0 and 3–7 days earlier were correlated with $\delta^{13}\text{C}_{\text{ER}}$, with the 5-day lag being significant (P value <0.05). To examine direct relationships between $\delta^{13}\text{C}_{\text{ER}}$

and recent G_s , we used models relating isotope discrimination to stomatal conductance and photosynthetic capacity at the leaf level to estimate values of stomatal conductance (“ G_{s-I} ”) that would be expected if respired CO_2 were derived entirely from recent photosynthate. We compared these values with estimates of G_s using direct measurement of transpiration at multiple locations in the watershed. Considering that the approach based on isotopes considers only the effect of photosynthetic discrimination on $\delta^{13}\text{C}_{\text{ER}}$, the magnitude and range in the two values were surprisingly similar. We conclude that: (1) $\delta^{13}\text{C}_{\text{ER}}$ is sensitive to variations in weather, and (2) $\delta^{13}\text{C}_{\text{ER}}$ potentially could be used to directly monitor average, basin-wide variations in G_s in complex terrain if further research improves understanding of how $\delta^{13}\text{C}_{\text{ER}}$ is influenced by post-assimilation fractionation processes.

Keywords Douglas-fir · Respiration · Cold air drainage · Soil moisture · Vapor pressure deficit

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Introduction

Complex terrain presents formidable challenges to ecosystem studies. Valleys, steep slopes, and windswept ridges impose wide variations in microclimate, soil properties, and plant communities; this variability greatly hinders strategies for systematic sampling and up-scaling. Furthermore, atmospheric properties in topographically complex regions impede ecosystem-scale flux measurements (Aubinet et al. 2003; Black et al. 1996; Lavigne et al. 1997; Paw et al. 2004; Staebler and Fitzjarrald 2004). Yet, many of the most carbon-rich and dynamic landscapes in the world occur in forested mountainous regions.

We noted from recent reports that the very conditions that complicate many ecosystem studies in mountainous regions may offer an opportunity for an entirely different approach to study processes at the ecosystem scale (Pypker et al. 2007a, b). In a deeply incised watershed (20–33° slopes) in western Oregon, we estimated that more than 90% of the respired CO₂ from the entire ecosystem can be flushed from the basin by nocturnal cold-air drainage (Pypker et al. 2007b). Such drainage in this watershed is persistent, occurs on more than 80% of summer nights, and is well mixed (Pypker et al. 2007a). Hence, nighttime air samples collected from the base of the watershed contain a representative sample of respired CO₂ from most or all of the watershed (Pypker et al. 2007b). We also found that, on most clear nights, the range of CO₂ concentrations over a single night is sufficient for using the Keeling plot approach to determine the carbon isotopic composition of ecosystem respiration ($\delta^{13}\text{C}_{\text{ER}}$) (Pypker et al. 2007a). Thus, we suggested the possibility of using $\delta^{13}\text{C}_{\text{ER}}$ in mountainous ecosystems as a tool to monitor seasonal and interannual variations in physiological processes in vegetation.

Previous work in our experimental watershed, as well as at many other sites, has demonstrated substantial seasonal variation in $\delta^{13}\text{C}_{\text{ER}}$ —sometimes exceeding 4‰ over the course of a few months (Bowling et al. 2002; Knohl and Buchmann 2005; Lai et al. 2005; Mortazavi et al. 2005; Ometto et al. 2002). Many previous studies have shown correlations between $\delta^{13}\text{C}_{\text{ER}}$ and environmental variables that are consistent with the ways that these variables affect carbon isotope discrimination during photosynthesis. For example, studies have shown that the carbon isotope composition of respired CO₂ becomes enriched in ¹³C (i.e., decreased isotopic discrimination) with increasing vapor pressure deficit (VPD) (Bowling et al. 2002; Knohl and Buchmann 2005; Mortazavi et al. 2005), decreased precipitation (Ometto et al. 2002), decreased soil moisture content (Fessenden and Ehleringer 2002; Lai et al. 2005; Ponton et al. 2006), and increased soil temperature (McDowell et al. 2004b). All of these environmental conditions are associated with reduced stomatal conductance, which in turn should result in decreased isotopic discrimination (Farquhar et al. 1989; Madhavan et al. 1991).

Another important prior finding comes from time-lagged analyses of the relationships between $\delta^{13}\text{C}_{\text{ER}}$ and VPD. Correlations between the two variables tend to be high when measured concurrently; correlations between VPD measured 1 or 2 days earlier than $\delta^{13}\text{C}_{\text{ER}}$ are lower, and then increase again with a lag of 3–5 days (Alstad et al. 2007; Bowling et al. 2002; Knohl and Buchmann 2005; Mortazavi et al. 2005). Although this pattern could arise from a periodicity in weather patterns, it is also consistent

with the notion that foliar and soil respiration, respectively, account for the two peaks, and that the lag time is a measure of the time it takes for carbon transport through phloem to the roots and subsequent consumption by growing root tips and microbes. The finding is also consistent with recent experiments that have demonstrated that more than 50% of below-ground respiration is derived from recently fixed carbon (Högberg et al. 2008; Högberg and Read 2006) and that the isotopic composition of soil respiration is strongly influenced by recent manipulations of above-ground components (Högberg et al. 2001).

The implications of these findings are profound, because a substantial component of the carbon in the ecosystem respiration flux, both above and belowground, appears to consist of carbon fixed 0–5 days earlier. Granted, these are over simplifications, as past work can only account for a fraction of the variability in $\delta^{13}\text{C}_{\text{ER}}$. A substantial portion of the remaining variability in the $\delta^{13}\text{C}_{\text{ER}}$ may result from the metabolizing of older carbon and/or fractionation during respiration (Duranceau et al. 1999, 2001; Ghashghaie et al. 2003, 2001; Hymus et al. 2005; O’Leary 1981; Prater et al. 2006; Xu et al. 2004). Despite the uncertainty, we hope that measurements of $\delta^{13}\text{C}_{\text{ER}}$ could one day be used to infer seasonal variations in canopy physiology.

Here, we represent one step toward this goal. We ask, to what degree do variations in $\delta^{13}\text{C}_{\text{ER}}$ describe variations in canopy-average stomatal conductance? Although several previous studies have shown correlations between $\delta^{13}\text{C}_{\text{ER}}$ and environmental factors, the purely statistical correlations do not account for the known kinetics of isotope discrimination in relation to leaf conductance. If canopy-average stomatal conductance can be predicted using $\delta^{13}\text{C}_{\text{ER}}$, it would suggest that the fractionation that occurs after carbon fixation is small or offset by other factors.

Our approach employed many simplifying assumptions. We characterized “average” environmental conditions in a heterogeneous watershed through measurements in a transect of eight plots, and estimated canopy-average stomatal conductance (G_s) using sapflow measurements of the dominant species, Douglas-fir [*Psuedostuga menziesii* (Mirb.) Franco] (which accounts for more than 90% of the tree basal area in the watershed). We used the measured $\delta^{13}\text{C}_{\text{ER}}$ to derive the stomatal conductance that would be expected if respired CO₂ were derived entirely from recent photosynthate (G_{s-1}); we did not account for possible variations in ambient CO₂ concentration, fractionation during respiration, isotopic composition of ambient CO₂ and temperature. These simplifications must be considered when interpreting the results. Nevertheless, the approach provides a step forward from simple correlations between environmental drivers and $\delta^{13}\text{C}_{\text{ER}}$.

Materials and methods

Study site

The study area was a 96-ha watershed (Watershed 1), located in the H J Andrews Experimental Forest in the western Cascades of central Oregon, USA (44.2°N, 122.2°W) (Fig. 1). The watershed, a steeply sloping V-shaped valley, is predominately covered by young Douglas-fir replanted following clear-cut harvesting in the late 1960s. Smaller components of the tree basal area consist of western hemlock [*Tsuga heterophylla* (Raf.) Sarg], big leaf maple (*Acer macrophyllum* Pursh), vine maple (*Acer circinatum* Pursh) and red alder (*Alnus rubra* Bong.); the angiosperm populations are greatest within the riparian area (Moore et al. 2004). The canopy was between 25 and 28 m tall (Moore et al. 2004). The site has wet mild winters and warm dry summers with a mean annual precipitation of 2,300 mm (Rothacher et al. 1967). In the warm dry summers, cold air drainage occurs on most nights (Pypker et al. 2007a). The soil has Andic properties and has a loamy to gravelly clay loam texture (Swanson and James 1975).

Environmental variables

A 37-m tower located at the base of the watershed was instrumented in May 2005 with ten shielded thermistors

(Model 107 temperature probe, Campbell-Scientific Inc., Logan, UT, USA), a net radiometer (Q7 REBS, Campbell-Scientific Inc.), a shielded temperature/RH probe (HMP45c, Campbell-Scientific Inc.), eight 2-D sonic anemometers (WS425, Vaisala, Helsinki, Finland), and two 3-D sonic anemometers (81000, RM Young, Traverse City, MI, USA). For most periods, the 2-D and 3-D sonic anemometers were measured at 0.1 and 1 Hz, respectively, and values were averaged over 1-min intervals. The remaining instruments were recorded every 1 s and averaged over 15-min intervals (using CR10X and CR23X data loggers, Campbell-Scientific Inc.).

Perpendicular to the axis of the valley, a transect of eight plots (four on each slope) were established in the spring of 2005 (Fig. 1). At each plot, air temperature and relative humidity were measured at mid-canopy (HMP45c, Campbell-Scientific Inc.), and soil moisture (Echo-20, Decagon Devices, Pullman, WA, USA) and soil temperature (Model 107 temperature probe, Campbell-Scientific Inc.) were measured at 5, 30, and 100 cm depth. Calibration equations that were specific to HJA soils were used to convert the millivolt signal from the soil moisture sensors to volumetric water content (Czarnomski et al. 2005). Observations were recorded by a data logger (CR23X, Campbell-Scientific Inc.) every 15 s and averaged over 15-min intervals.

Sample collection and analysis

On 21 nights, from 13 July to 5 October 2005 and 8 May to 25 October 2006, air samples for isotope analysis were collected using an automated air collection system (Hauck 2006) at three heights (3, 10, 30 m) on the tower (Fig. 1). Air was pumped at $\sim 1.5 \text{ l min}^{-1}$ (Model # UNMP30KNDC, KNF Neuberger, Inc.) through tubing from one of the three heights, as selected by a six-position, stainless-steel valve (Valcon M, Valcon Instruments, Houston, TX, USA), then passed through a 200-cc cylinder containing magnesium perchlorate to absorb water vapor before entering a 16-position sample valve (Valcon M, Valcon Instruments). At each position on the valve, a 15-ml coiled sample loop of stainless steel was connected, so that sampled air passed through one of the 16 loops prior to exiting. The air was then directed to an infra-red gas analyzer (IRGA) (LI-6252, LI-COR Inc., Lincoln, NE, USA). The valves, pumps and IRGA were controlled by a data logger (CR10X, Campbell-Scientific Inc.), which recorded the sampling time and CO_2 concentration of the air passing through the system.

Sampling began 1 h after sunset, at 5-min intervals. To ensure a broad range of CO_2 concentrations, the samples were collected only if their concentrations differed from previous samples by a value set a priori (Lai et al. 2005). If the concentration of CO_2 in the current sample loop

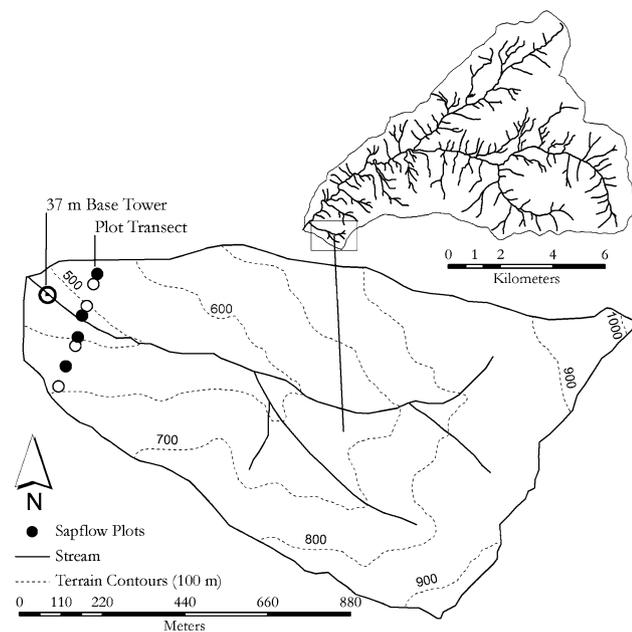


Fig. 1 Map of the watershed located in the HJ Andrews Experimental Forest. The map shows the locations of the 37-m base tower and the eight research plots along the transect that were used in this study. The dark circles represent the four plots where sapflow was monitored

exceeded that of past samples by more than 4 ppm, the valve moved to the next position and a new sampling height on the tower was selected. If the concentration difference was 4 ppm or less, the system continued to sample from the same tower height and sample valve position and attempted to take a sample 5 min later. This process continued until either all 15 sample loops filled (the 16th sample loop could not be used to collect a sample because it remained open to the atmosphere after the system finished collecting samples) or it was 1 h before sunrise. If the sample loops were full, the system shut down. If they were not full 1 h prior to sunrise, the system collected samples every 5 min until the remaining sample loops were filled (Hauck 2006).

Air in the sample loops was analyzed in the laboratory within 24 h of collection. For $\delta^{13}\text{C}$ analysis, we used a Finnigan/MAT DeltaPlus XL isotope ratio mass spectrometer interfaced to a GasBench II automated headspace sampler at the College of Oceanic and Atmospheric Sciences, Oregon State University (Hauck 2006). The GasBench-II is a continuous flow interface that allows injections of several aliquots of a single gas sample into a mass spectrometer for automated isotope determinations of small gas samples. The sample valve was directly plumbed into the mass spectrometer. Helium pushed the sample air out of the sample loops and into the mass spectrometer. A typical analysis consisted of three gas standards (tank CO_2 –He mixtures), five sample replicates and an additional two gas standards for every sample loop. The ratio of heavy to light isotopes of each sample (R) was related to the Vienna PeeDee Belemnite standard (R_{std}) in order to express the carbon isotope ratios as $\delta^{13}\text{C}$ (‰).

$$\delta = \left(\frac{R}{R_{\text{std}}} - 1 \right) 1000 \quad (1)$$

The CO_2 concentration [CO_2] of each sample was calculated from the peak volt area produced by the mass spectrometer analysis of each sample loop (Hauck 2006). To calibrate the system, each loop was filled with a gas of a known CO_2 concentration (403 and 958 ppm; as determined by NOAA). The air was passed from the loops into the mass spectrometer in the same manner as the air samples from the field. A linear relationship between peak volt area and CO_2 concentration was generated. This CO_2 calibration was performed independently for each sampling date.

Data analysis

A two-end-member mixing model (Keeling plot) was used to estimate $\delta^{13}\text{C}_{\text{ER}}$ (Keeling 1958). Prior to inclusion in the Keeling plot, all data points were screened for accuracy. If the standard deviation in the $\delta^{13}\text{C}$ of the reference gas or

sample gas was greater than 0.2, the point was removed. The $\delta^{13}\text{C}$ values of the air samples from the sample loops were plotted against their $1/[\text{CO}_2]$ values. Similar to Zobitz et al. (2006), our statistical analysis demonstrated that ordinary least squares regression was the best method for our data set (Z. Kayler, unpublished data). Standard errors of the intercepts were estimated using a bootstrap method. After the Keeling plot was generated, the regression was visually checked for non-random residuals. If non-random residuals were found in the Keeling plot, data collected on that date were not used in the analysis. Data from only two dates (24 April 2006 and 31 July 2006) were removed by this protocol.

The relationships between $\delta^{13}\text{C}_{\text{ER}}$ and each of average midday VPD, average soil matrix potential (ψ_{m}) at 100 cm and G_{s} were explored using correlation analysis. Changes in $\delta^{13}\text{C}_{\text{ER}}$ were regressed against measured midday VPD measured at eight locations in the watershed and modeled midday G_{s} from 0 to 8 days earlier. Time-lag analysis was used to determine whether there was a delay between when the carbon was fixed and when it was respired. In contrast, $\delta^{13}\text{C}_{\text{ER}}$ was only regressed against ψ_{m} measured only on the same day because, during the dry season, ψ_{m} is highly correlated to ψ_{m} measured on adjacent days.

Soil matrix potential (ψ_{m})

To determine whether there was a relationship between soil water content and $\delta^{13}\text{C}_{\text{ER}}$, we estimated ψ_{m} in the watershed by relating the volumetric soil water content at 100 cm (θ) and pre-dawn leaf water potential measured along the transect (data not shown). Tri-weekly, water potentials of three small detached twigs were measured in four of the transect plots 1–3 h prior to sunrise using a field portable pressure chamber (PMS systems, Corvallis, OR, USA). It was assumed that the pre-dawn water leaf potential was equivalent to the (ψ_{m}) of the plot. It is generally assumed that leaf water potential equilibrates at night to that region of soil with the highest (least negative) water potential (Richter 1997). Problems with the technique (Donovan et al. 2001; Kavanagh et al. 2007) suggest that the pre-dawn measurements are unlikely to exactly represent the soil matrix potential at the 100 cm depth. Therefore, one must be cautious when interpreting the results. Soil matrix potential was estimated using:

$$\Psi_{\text{m}} = \frac{-0.43\theta_{100\text{cm}}}{(-7.6 + \theta_{100\text{cm}})} \quad (2)$$

where ψ_{m} was assumed to be equal to the measured pre-dawn water potential and $\theta_{100\text{cm}}$ is the volumetric water content at 100 cm ($R^2 = 0.96$; P value < 0.0001).

Transpiration–heat-dissipation method

From April to October 2006, we used heat-dissipation sensors (Granier 1985, 1987) to measure the water flux of ten trees per plot in four plots along the transect (Fig. 1). The trees measured were representative of the range of tree diameters in each plot. In each tree, a 2-cm long sensor was inserted into the xylem at the 0- to 2-cm depth interval at 1.4 m above ground. In three dominant trees per plot, 1-cm-long sensors were installed at two additional depth intervals (2–3 and 3–4 cm) to account for radial flux profiles (Phillips et al. 1996). Sapwood depth measurements, visually inspected from increment cores, indicated that none of the sensors crossed the heartwood boundary. Measurements were recorded by a data logger (CR23X, Campbell-Scientific Inc.) every 15 s and averaged over 15-min intervals. Measurements from all pairs of probes were converted to sap flux density ($\text{g H}_2\text{O m}^{-2}$ sapwood s^{-1}). In the inner (>2 cm depth) xylem of trees that were not equipped with inner sap flow probes, this value was estimated from the ratio between the outer 0- to 2-cm flux and the inner 2- to 3-cm or 3- to 4-cm fluxes from the measured trees. We assumed that there was no change in flux between the depth of the 3- to 4-cm sensor and the heartwood boundary.

We scaled measurements from individual sensors to mean transpired water flux per unit ground area (mm day^{-1}). First, for each plot, the diameter at 1.4 m was measured for all trees. Xylem depth for each tree was calculated using a diameter-to-xylem depth relationship developed from over 200 tree cores taken across the watershed (Woolley, unpublished data). For each tree, the total xylem area at each depth interval (0–2, 2–3, >3 cm) was calculated.

The flux within each depth interval of xylem was calculated as the product of the area of that interval and the measured or predicted flux; we then summed the fluxes for each xylem depth interval to estimate total flux per tree. Last, we summed the fluxes of all the trees on each plot and divided by the ground area to estimate mean water flux per unit ground area.

Estimates of canopy-average stomatal conductance (G_s and G_{s-1})

To test the feasibility of simulating the response of canopy-average stomatal conductance to changing environmental variables using $\delta^{13}\text{C}_{\text{ER}}$ measurements, we estimated canopy-average stomatal conductance in two ways: using sapflow measured at four plots in the watershed (Fig. 1); and by calculating the canopy-average stomatal conductance necessary to produce the $\delta^{13}\text{C}_{\text{ER}}$ measured on the tower using models described by Farquhar et al. (1989, 1980).

We used daily sapflux data collected at four locations within the watershed to predict midday canopy-average stomatal conductance (G_s) in 2006 (Fig. 1). For days of the year 96–304, we estimated G_s for each plot using:

$$G_s = \frac{E}{\text{VPD} \times s} \quad (3)$$

where E represents transpiration (mol day^{-1}) and s the number of seconds of daylight for each day (s day^{-1}); midday VPD is expressed as mol mol^{-1} . The number of seconds of daylight per day at the research location was estimated using equations described by Campbell and Norman (1998).

We also used values of $\delta^{13}\text{C}_{\text{ER}}$ to estimate a hypothetical value for canopy-average stomatal conductance that would be expected if recent photosynthate were the only source of carbon in ecosystem respiration (G_{s-1}). To do this, we assumed that the difference between $\delta^{13}\text{C}_{\text{ER}}$ and δ_a (the isotopic ratio of the source air) is equal to photosynthetic discrimination $\delta^{13}\text{C}_{\text{ER}}$, and we employed biophysical models for leaf-level photosynthetic isotopic discrimination (Farquhar et al. 1989) and carbon assimilation/internal CO_2 curves (Farquhar et al. 1980). First, carbon discrimination ($\delta^{13}\text{C}_{\text{ER}}$) was estimated using:

$$\Delta^{13}\text{C}_R = \frac{(\delta_a - \delta^{13}\text{C}_R/1000)}{(1 + \delta^{13}\text{C}_R/1000)} 1000 \quad (4)$$

where δ_a is the isotopic ratio of the source air (Buchmann et al. 1998). We assumed δ_a to be -8.2‰ in 2005 and 2006. Then, the mean internal CO_2 concentration [C_i] within the leaf was estimated using:

$$C_i = \frac{(\Delta^{13}\text{C}_R - a)}{(b - a)} \quad (5)$$

where a is the fractionation due to the diffusion of CO_2 into the stomata and b is the net fractionation due to carboxylation, taken as -4.4 and -27.7‰ , respectively (Farquhar et al. 1989). Based on the C_i values, the assimilation rate (A) of CO_2 was estimated using:

$$A = \left(\frac{V_{\text{cmax}} C_i / 10}{(C_i / 10 + K_c (1 + O_x / K_o))} \right) \left(1 - \frac{0.5 O_x}{\tau C_i / 10} \right) - R_d \quad (6)$$

where V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) is the maximum rate of Rubisco-mediated carboxylation, K_c and K_o ($\mu\text{mol mol}^{-1}$) are respectively the Michaelis–Menten coefficients for CO_2 and O_2 binding to Rubisco, O_x ($\mu\text{mol mol}^{-1}$) is the intercellular partial pressure of oxygen, τ ($\mu\text{mol mol}^{-1}$) is the CO_2 compensation point, and R_d ($\mu\text{mol m}^{-2} \text{s}^{-1}$) is the daytime respiration rate. Values of V_{cmax} , τ and R_d were estimated from A/C_i curves measured on Douglas-fir trees of similar age and needle nitrogen concentration at Wind River, Washington (Bond et al., unpublished data). These

values are 46, 27.5, and 42, respectively. The variables K_c , K_o , and O_x were treated as constants with assumed values of 21, 2.32, and 1.5, respectively. We ignored potential temperature effects on K_c , K_o , O_x , and τ . Canopy-average stomatal conductance for water vapor was then estimated using:

$$G_{s-I} = \frac{A}{(C_a - C_i)} 1.6 \quad (7)$$

where C_a is the atmospheric concentration of CO_2 (assumed to be $380 \mu\text{mol mol}^{-1}$), and the factor 1.6 converts from CO_2 conductance to water vapor conductance (Taiz and Zeiger 1991). It should be noted that the estimates of G_{s-I} using Eqs. 4–7 rely on measurements at both the canopy scale (e.g., $\delta^{13}\text{C}_{\text{ER}}$) and at the leaf scale (sun foliage) (e.g., V_{cmax} , τ , K_c , K_o , R_d and O_x).

For leaf-scale variables, we used kinetic parameters for the photosynthetic capacity of sun foliage of the dominant species, Douglas-fir, which constitutes more than 70% of the basal area in our experimental basin (Moore et al. 2004). Carbon isotope discrimination on the ecosystem scale is “flux weighted”. In other words, foliage that has the highest carbon assimilation also has the greatest influence on photosynthetic discrimination. As much as 80% of net carbon assimilation in a dense forest occurs in the sun foliage (Bond et al. 1999) as the majority of the solar radiation is absorbed by the upper portion of the canopy (Parker et al. 2002). We did not consider understory species or even shade foliage of Douglas-fir because their contribution to total ecosystem carbon assimilation is small.

Results

Seasonal $\delta^{13}\text{C}$ of ecosystem respiration ($\delta^{13}\text{C}_{\text{ER}}$)

In 2005, $\delta^{13}\text{C}_{\text{ER}}$ of ecosystem respiration ranged between -26.3 and -25.9‰ from 13 July to 10 August 2005 (Fig. 2). During subsequent sampling dates, $\delta^{13}\text{C}_{\text{ER}}$ increased to greater than -23.3‰ ; but after 21 September 2005, $\delta^{13}\text{C}_{\text{ER}}$ began to decrease again. In 2006, $\delta^{13}\text{C}_{\text{ER}}$ followed a similar pattern, with the $\delta^{13}\text{C}_{\text{ER}}$ increasing in the summer months and then declining later in the year (Fig. 2). However, unlike 2005, the rise and fall of $\delta^{13}\text{C}_{\text{ER}}$ began earlier; by late July, $\delta^{13}\text{C}_{\text{ER}}$ rose to -24.0‰ and, by early September, $\delta^{13}\text{C}_{\text{ER}}$ began to decrease.

Environmental variables

Volumetric soil moisture (θ) and midday VPD responded to the seasonal summer dry period (Fig. 3). As the summer progressed, VPD increased and θ decreased. However,

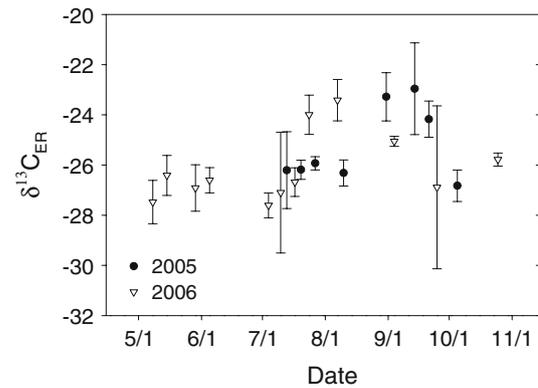


Fig. 2 Seasonal changes in $\delta^{13}\text{C}_{\text{ER}}$, as measured at tower located at the base of ~ 45 -year-old Douglas-fir watershed in 2005 and 2006. The error bars represent the standard errors of the Keeling plot intercepts

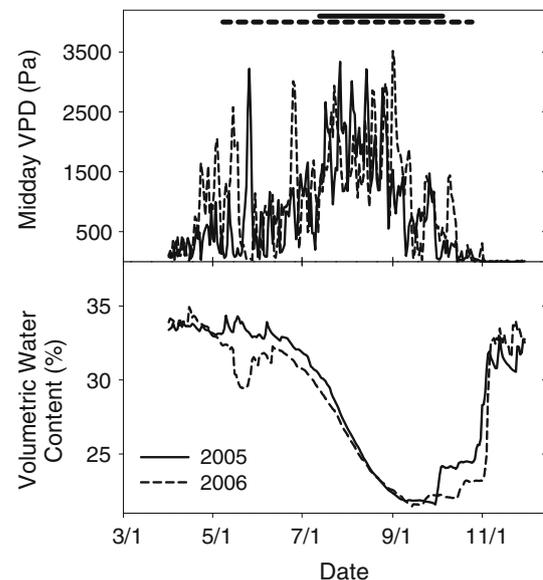


Fig. 3 Mean midday vapor pressure deficit (VPD) within the forest canopy and mean soil moisture conditions at 100 cm depth in a Douglas-fir watershed in 2005 and 2006. The measurements are the average from eight locations along a transect (Fig. 1). The SE for the eight plots averaged 33 and 2.9 for the VPD and soil moisture measurements, respectively. The solid and dashed lines at the top of the graphs represent the range of sample dates in 2005 and 2006, respectively

there were differences between 2005 and 2006. In 2006, θ decreased more rapidly in the spring and recovered more slowly in the fall than in 2005.

Correlation between $\delta^{13}\text{C}_{\text{ER}}$ and environmental variables

Changes in $\delta^{13}\text{C}_{\text{ER}}$ were either weakly or significantly correlated with VPD, ψ_m and G_s . Midday VPD values measured 0–7 days were weakly correlated to $\delta^{13}\text{C}_{\text{ER}}$

(Fig. 4a), with the correlation to the 0-day lag being the strongest ($R^2 = 0.13$; P value < 0.12). Periodicity in the synoptic weather patterns was not apparent in the data set when VPD was compared with itself over an 8-day lag (data not shown). Midday VPD correlated most closely with that measured on adjacent days, and correlation strength steadily declined as the difference in time increased (data not shown). $\delta^{13}\text{C}_{\text{ER}}$ and ψ_{m} were significantly correlated, with $\delta^{13}\text{C}_{\text{ER}}$ increasing as the soil matric potential became more negative ($\psi_{\text{m}} < -0.6$ MPa) ($R^2 = 0.44$; P value < 0.002) (Fig. 5). However, it is difficult to determine whether the correlation is purely the result of the drying soil, because the periods with the most negative soil matric potential occurred when VPD was also the highest (Fig. 3). The reliability of the proposed correlation is further questioned because nocturnal transpiration could have made the ψ_{m} appear more negative. However, the trees were becoming stressed as the summer progressed, as changes in $\delta^{13}\text{C}_{\text{ER}}$ were inversely correlated with G_{s} derived using the heat-dissipation method (Fig. 4b). The correlations were strongest when G_{s} lagged in time by 5 days ($R^2 = 0.56$; P value < 0.05). The changes in $\delta^{13}\text{C}_{\text{ER}}$ also correlated weakly with midday G_{s} from 0, 3 and 4 days earlier ($R^2 > 0.26$; P value < 0.14) (Fig. 4b).

Prediction of canopy-average stomatal conductance using $\delta^{13}\text{C}_{\text{ER}}$

Values of average midday G_{s} from 3 to 5 days earlier, as estimated using the heat-dissipation method, ranged between 0.07 and $0.18 \text{ mol m}^{-2} \text{ s}^{-1}$ (Fig. 6). When $\delta^{13}\text{C}_{\text{ER}}$ was used to estimate $G_{\text{s}-1}$ using the models of Farquhar et al., the results were significantly correlated with the average midday stomatal conductance measured 3–5 days earlier using the heat-dissipation method (P value < 0.05 ; $R^2 = 0.51$) (Fig. 6). The regression line was slightly offset from the one-to-one line, with $G_{\text{s}-1}$ larger than G_{s} .

Discussion

Correlations of $\delta^{13}\text{C}_{\text{ER}}$ with changes in environmental variables

In this study, $\delta^{13}\text{C}_{\text{ER}}$ was weakly correlated with average midday VPD 6 days earlier and significantly correlated with concurrent ψ_{m} (Figs. 4a, 5). (We did not conduct lag analyses of $\delta^{13}\text{C}_{\text{ER}}$ versus ψ_{m} because the autocorrelation of ψ_{m} is very strong for any sequence of days.) The sensitivity of $\delta^{13}\text{C}_{\text{ER}}$ to changes in soil moisture has been demonstrated by others (Fessenden and Ehleringer 2002;

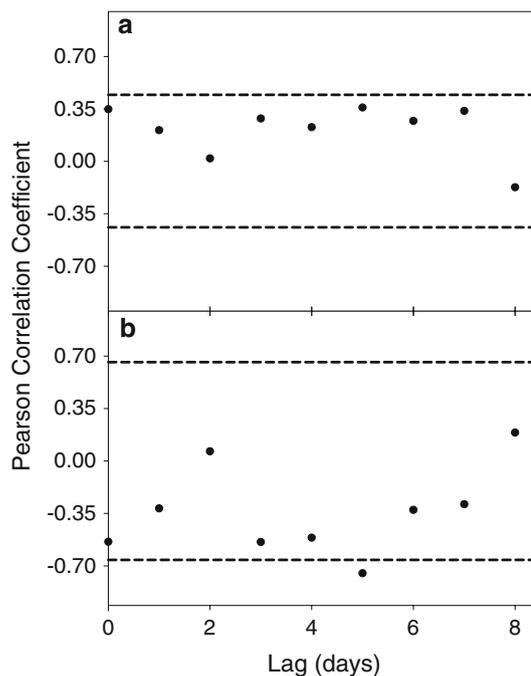


Fig. 4 Lag correlation analysis between $\delta^{13}\text{C}_{\text{ER}}$ and average midday vapor pressure deficit (VPD; **a**), as measured at eight locations along a transect in 2005 and 2006 (Fig. 1), and midday canopy conductance (G_{s} ; **b**), as derived from the heat-dissipation method in 2006. The dashed lines indicate the R required for the correlation to be statistically significant (P value < 0.05)

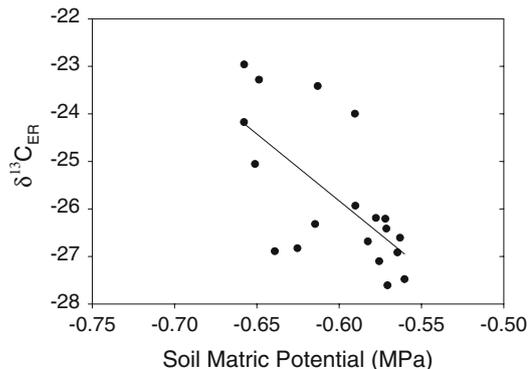


Fig. 5 Relationship between mean soil matric potential at 100 cm (ψ_{m}) from the plots along the transect and $\delta^{13}\text{C}_{\text{ER}}$ in 2005 and 2006. The solid black line represents the statistically significant regression between the two variables ($R^2 = 0.44$; P value < 0.002)

Lai et al. 2005; Ponton et al. 2006). The results are also consistent with the physiology of Douglas-fir trees. Bond and Kavanagh (1999) demonstrated that leaf-level stomatal conductance of Douglas-fir declined once soil matric potential dropped below -0.5 MPa. This decline should result in an enrichment of ^{13}C in the photosynthate. However, the strength of the correlation between ψ_{m} and $\delta^{13}\text{C}_{\text{ER}}$ can be questioned because the predawn measurements may have been affected by nocturnal transpiration

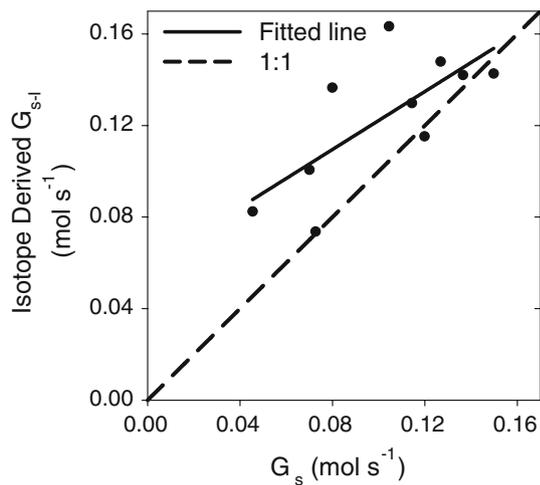


Fig. 6 The significant correlation between the average midday canopy conductance using the heat-dissipation method (x -axis) (G_s —from 3 to 5 days earlier) and the G_{s-I} derived from $\delta^{13}C_{ER}$ in combination with physiological models (y -axis) (Farquhar et al. 1989, 1980) ($R^2 = 0.51$; P value < 0.05). The *dashed line* represents the 1:1 line. Two isotope sample dates were not included because the corresponding sapflow data was missing from one or more plots (8 May and 5 June 2006)

(Donovan et al. 2001; Kavanagh et al. 2007). The change in $\delta^{13}C_{ER}$ may have been responding to the combined effect of lower ψ_m and higher VPD. Changes in $\delta^{13}C_{ER}$ appeared to track annual differences in the patterns of VPD and ψ_m in 2005 and 2006.

$\delta^{13}C_{ER}$ became enriched in ^{13}C earlier in the season in 2006 than in 2005. This earlier shift corresponded with a more rapid decline in soil moisture in 2006 relative to 2005 (Fig. 3), suggesting that the difference in water availability may have influenced $\delta^{13}C_{ER}$. The range of $\delta^{13}C_{ER}$ was similar to those reported in previous studies of Douglas-fir (Bowling et al. 2002; Fessenden and Ehleringer 2002; Lai et al. 2005; Ponton et al. 2006; Pypker et al. 2007a).

Previous research has emphasized the correlations between $\delta^{13}C_{ER}$ and VPD (Bowling et al. 2002; Knohl and Buchmann 2005; Mortazavi et al. 2005) or soil moisture (Lai et al. 2005; Ponton et al. 2006). Both of these environmental variables may have a strong (and interacting) influence on stomatal conductance, which in turn affects photosynthetic isotope discrimination (Farquhar et al. 1989). These correlations suggest that the respired CO_2 was recently fixed and that discrimination during photosynthesis has considerable influence on the isotopic signature of respiration, although there can be alternative explanations (e.g., direct effects of soil moisture on carbon sources used by microbes).

Similar to the results in our study, McDowell et al. (2004b) found that both soil water content and VPD governed variations in $\delta^{13}C_{ER}$ for a Douglas-fir forest in the Coast Range of Oregon. VPD exerted a stronger influence

in their study than in ours, perhaps because soil moisture tended to be high for most of their measurement periods.

In 2005 and 2006, we monitored $\delta^{13}C_{ER}$ from 13 July to 25 October and from 8 May to 26 October, respectively, thus obtaining measurements over a wider range of soil moisture and VPD (Figs. 3, 4) values relative to a previous pilot study in this watershed (Pypker et al. 2007a). We suspected that the longer measurement period might produce stronger correlations between $\delta^{13}C_{ER}$ and environmental variables than our previous pilot study; at H.J. Andrews LTER, there is minimal rainfall between July and mid-September (Pypker et al. 2006; Rothacher et al. 1967). However, even with the wider range of VPD and soil moisture, only changes in soil moisture correlated significantly with changes in $\delta^{13}C_{ER}$.

Care must be taken when interpreting the results because changes in VPD or soil moisture do not directly cause isotope discrimination. Instead, these variables affect stomatal conductance through species-specific biophysical signals. The relationships between G_s and VPD or soil moisture are non-linear and are mutually interdependent (Bond and Kavanagh 1999; Marshall and Waring 1984; Pezeshki and Hinckley 1982; Waring and Schlesinger 1985). Thus, when using linear models to correlate environmental variables to $\delta^{13}C_{ER}$, one may misinterpret how changes in a given variable will influence changes in $\delta^{13}C_{ER}$ because the analysis assumes the relationship is linear. Since stomata control the supply of CO_2 to the leaf, stomata affect the ratio ^{12}C to ^{13}C fixed during photosynthesis by altering the amount of CO_2 available for fixation. Thus, stomatal conductance may be a better variable than ψ_m or VPD to use when conducting lag correlation analysis. The $\delta^{13}C_{ER}$ measured at the tower correlated significantly with midday G_s (Fig. 4b).

Can $\delta^{13}C_{ER}$ be used to predict G_s ?

The significant association between $\delta^{13}C_{ER}$ and modeled G_s suggests that with some improved understanding, $\delta^{13}C_{ER}$ measurements might be used to predict the average response of stomata within an entire watershed to environmental stressors. Indeed, when the Farquhar equations in combination with the measured $\delta^{13}C_{ER}$ were used to estimate G_{s-I} , the estimates of G_s and G_{s-I} were significantly correlated (Fig. 6). Perhaps more important, the estimates fall near the 1:1 line. In general, the isotope-derived estimates of G_{s-I} were larger than those derived from sapflow, and the difference appeared to increase as G_s increased. This could be due to the influence of shade foliage on isotope discrimination, seasonal shifts in above-ground versus below-ground carbon allocation, shifts in the use of current versus old carbon by soil microbes, or to a myriad of other possibilities we did not consider here. The

fact that the values of G_s and G_{s-I} are similar, despite the many reasons they could differ, suggests that recently fixed carbon has an overwhelming influence on ecosystem respiration. Further research is needed, but these results suggest that $\delta^{13}C_{ER}$ measurements may be used to estimate G_s when other processes are better understood.

Our study suggests important avenues for future research. For example, fractionation during autotrophic respiration is one of the several important additional influences to $\delta^{13}C_{ER}$. Recent research suggests that during dark respiration, leaf mesophyll cells frequently switch the substrate they are metabolizing (e.g., from glucose to lipids) and alter the proportion of sucrose being converted to CO_2 and lipids (Ghashghaie et al. 2003). As the portion of respired CO_2 that is derived from lipids increases, the ^{13}C content of the respiration decreases; likewise, increased production of lipids during the respiratory process also increases the ^{13}C content of the respiration. The magnitude of this “apparent fractionation” (Ghashghaie et al. 2003) may depend on both the species and the environmental variables (Ghashghaie et al. 2003, 2001). Regardless of the mechanism for discrimination during respiration, past research has demonstrated that the difference between the $\delta^{13}C$ of the sucrose in the leaf and the respired CO_2 changes as the night progresses (Barbour et al. 2007; Mortazavi et al. 2006) and ranges between ± 0 and 10‰ (Duranceau et al. 1999, 2001; Ghashghaie et al. 2003, 2001; Hymus et al. 2005; O’Leary 1981; Prater et al. 2006; Xu et al. 2004). Therefore, the effect of discrimination during respiration on the ^{13}C of ecosystem respiration likely exists for the forest in this study, but it could range from being nominal to greater than 10‰. The respiratory fractionation for Douglas-fir trees has not been reported and, therefore, requires further investigation.

Fractionation post-photosynthesis scales to the ecosystem level as past research demonstrates that different carbon pools and fluxes have different isotopic ratios (see review by Bowling et al. 2008). The isotopic composition of leaf sugars are typically close to $\delta^{13}C_{ER}$, but other components within the ecosystem that respire CO_2 can be very different from each other (Bowling et al. 2008). For example, coarse woody debris tends to be more enriched than the isotopic composition of the bulk leaf (Hobbie et al. 2001; Kohzu et al. 1999). The isotopic composition of respiration from the different components can also differ from $\delta^{13}C_{ER}$. For example, the isotopic composition of the respiration from leaves tend to be more enriched than $\delta^{13}C_{ER}$ (Bowling et al. 2002, 2003; McDowell et al. 2004a; Mortazavi et al. 2005), but more depleted than respiration from roots (Badeck et al. 2005; Klumpp et al. 2005). Furthermore, the $\delta^{13}C_{ER}$ is frequently not in mass balance as leaf respiration and soil respiration because both tend to be more enriched relative to $\delta^{13}C_{ER}$ (McDowell et al.

2004a). All of these differences will contribute to the $\delta^{13}C_{ER}$, thereby affecting the ability to use the biophysiological equations we used to estimate assimilation rates or G_s . Clearly, more research is needed to understand how the $\delta^{13}C$ signal is altered by all components of the carbon cycle.

Another important area for future research is the more precise quantification of the contribution of recent photosynthate to $\delta^{13}C_{ER}$. Recent work suggests that greater than 50% of belowground respiration is derived from recent photosynthate (Högberg et al. 2008; Högberg and Read 2006); however, this proportion may vary seasonally or among different forest types. It is intriguing that the difference between G_s and G_{s-I} in our study was small when G_s was high, and that as conductance decreased, G_s was increasingly underestimated by G_{s-I} . A potential explanation for this finding is that recent photosynthate accounted for a smaller proportion of $\delta^{13}C_{ER}$ during times of low stomatal conductance, and that the contribution of older soil carbon to $\delta^{13}C_{ER}$ created a positive bias in G_{s-I} . It is also possible that in drier soil conditions, post-assimilation fractionation processes become more important. Differences between the results of our study and results reported by McDowell et al. (2004b) also suggest that the proportional contribution of heterotrophic respiration to $\delta^{13}C_{ER}$ may alter the relationships between $\delta^{13}C_{ER}$ and G_s . McDowell et al. (2004b) reported that in a ponderosa pine forest, VPD was inversely correlated to G_s , but there was no correlation between VPD or soil moisture and $\delta^{13}C_{ER}$. A potential explanation is that recent photosynthate accounted for a lower proportion of total ecosystem respiration in the pine forest than in the Douglas-fir forests of their study or of ours (LAI = 2.0 in the pine forest studied by McDowell et al. (2004b)—less than 1/3 of the LAI in the Douglas-fir forests of their study or ours), and as a result the relationship between $\delta^{13}C_{ER}$ and G_s was therefore not as strong.

Ecosystem studies that used $^{13}CO_2$ or $^{14}CO_2$ to trace the time required for photosynthate to travel from the leaves to the roots and be respired from the soil report that the transfer was rapid, with substantial portion of the recently fixed carbon exiting the soil in 1–4 days (Andrews et al. 1999; Ekblad et al. 2005; Ekblad and Högberg 2001; Howarth et al. 1994; Steinmann et al. 2004). Past research also reports a 56% reduction in soil respiration 5 days following tree girdling because the roots were not being supplied by recent photosynthate (Högberg et al. 2001). On the ecosystem scale, the significant correlation between changes in $\delta^{13}C_{ER}$ and environmental variables suggests that changes in $\delta^{13}C_{ER}$ result from recent weather, further supporting the assumption that a substantial portion of the respired CO_2 is from recent photosynthate (Alstad et al. 2007; Bowling et al. 2002; Knohl and Buchmann 2005; Lai et al. 2005;

McDowell et al. 2004b; Mortazavi et al. 2005; Ometto et al. 2002; Ponton et al. 2006). Therefore, past research on the plot and ecosystem scale demonstrates that a considerable portion of respired carbon results from recent changes in discrimination during photosynthesis.

Other important avenues for future research include: (1) quantification of temperature effects on A/C_i curves—in this study, we ignored potential temperature effects—and determination of the impact of these effects on the relationship between $\delta^{13}C_{ER}$ and G_{s-I} ; (2) evaluation of the importance of minor species in a forest to photosynthetic discrimination on an ecosystem scale—we considered physiological attributes of only the dominant species, the Douglas-fir; and (3) evaluation of the spatial variability in environmental variables in estimates of $\delta^{13}C_{ER}$ for the whole basin. Our study used sapflow and environmental measurements from eight small plots to derive an estimate of average G_s for the entire 96-ha basin, and we used this value as “truth” for comparison with G_{s-I} . We need to evaluate the potential impacts of spatial variability on the estimate of average G_{s-I} .

Despite all the assumptions and simplifications in our analyses, the estimates of G_{s-I} were close to G_s (Fig. 6), and when stomatal conductance was high, G_{s-I} and G_s were indistinguishable. Given all the simplifications and assumptions involved, the correlation between the two estimates may be fortuitous; however, we think the similarity between the two completely independent estimates of G_s is all the more striking given these many sources of potential deviation. The relationship compels further investigation.

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